

PHILIP MORRIS, INC.

RESEARCH CENTER

COMPLETION REPORT

THE QUANTITATIVE DETERMINATION
OF COUMARIN IN CIGARETTES

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I. INTRODUCTION AND SUMMARY

A new method for the quantitative determination of coumarin in tobacco filler has been developed. Heretofore methods for coumarin used either a colorimetric or an ultra-violet readout. These methods are not suited for the determination of low levels of coumarin in tobacco. The present fluorometric method developed is specific and sensitive.

Coumarin in tobacco is quantitatively steam distilled from an acidic, salt-saturated medium. The distillate is treated with a 1 N solution of KOH which hydrolyzes coumarin. Subsequent exposure of this solution to an ultra-violet source produces the fluorescent trans isomer.

Analyses of 17 brands of commercial cigarettes revealed coumarin levels as high as 182 ug per cigarette for Lucky Strike Filters to none detected for Reynold's products. The coumarin content of various commercial cigarettes is tabulated in Table 1.

II. FLUOROMETRIC DETERMINATION OF COUMARIN

Feigl's qualitative spot test¹ consists of reacting coumarin with a 5% caustic solution. The cleavage of the lactone ring produces the potassium salt of the cis isomer of o-hydroxy cinnamic acid. Ultra-violet radiation is used to convert the cis isomer into the fluorescent trans isomer.²

It was necessary to determine the conditions necessary to convert the qualitative method for coumarin into a quantitative method. Initial studies showed that the fluorescence of the anion was indeed a direct function of the amount of coumarin subjected to hydrolysis. Plots of Fluorescence vs. Concentration showed a linear relation which is of prime importance in a quantitative procedure.

Recovery of coumarin in the steam distillation step has been found to be essentially quantitative (about 95%) when done in the manner prescribed in the method. To facilitate use of existing equipment, it is necessary to use a sample size of 20 g or 20 cigarettes. The large sample size reduces variation due to sampling. The relative standard deviation at the two sigma level for one determination of a typical commercial sample is $\pm 12\%$. At least two samples per determination are recommended.

Coumarin can be successfully determined in filters in the absence of charcoal. In a special experiment it was shown that coumarin does drift into the filters although this drift may not account for all of the coumarin in the filter.³

A. Investigation of Conditions

The photolysis step of Feigl's method was found to require 30 minutes to develop maximum fluorescence. No decomposition of the sample was observed from exposure to ultra-violet for a period of several hours. An exposure time of 45 minutes for 5 samples in 150 ml beakers all under the same lamp has been found satisfactory. It was later determined that in 45 minutes, practically all of the background fluorescence disappears.

Reagent grade KOH is specified for hydrolysis to minimize introduction of impurities which can cause uncertainty in blank analysis. During this period the Turner Model 110 proved to be a very stable instrument with excellent day to day reproducibility.

The sodium chloride used in the steam distillation has not been found to be critical with respect to purity. Salt in the form of rock salt, table salt, and reagent grade has been used. Fifty pound bags of table salt are recommended both for purity and cost.

For the amount of coumarin encountered, a liter of distillate was found to be a sufficient volume to collect when distilling from an acidic, salt-saturated medium. If the need ever arose, the entire distilling apparatus could be scaled down to accomodate smaller sample sizes.³

B. Coumarin Recovery

A standard solution of coumarin in water was prepared and serial dilutions of the original standard were prepared to duplicate the concentration of coumarin that would be obtained after steam distillation. By comparing the fluorescence of these direct standards to the recovered steam distillates of known amounts of coumarin it was determined that 95% of the coumarin is recovered in the steam distillation step. Recovery of coumarin which was added to tobacco previously analyzed for coumarin gave recoveries within the limits of error of the method. It was found that recovery from carbon was poor (40%).

To simplify matters the standard curve used in the procedure is prepared from the fluorescence of standards recovered from distillation. This eliminates the need to know the exact amount recovered.

C. Coumarin in Filters

The coumarin content in filters of several brands of cigarettes was analyzed and the following results were obtained: L.S. Filter - 30 ug/cigt, Lark - 13 ug/cigt, Parliaments - 5 ug/cigt. The analysis of coumarin in a carbon filter is not quantitative since coumarin is not quantitative extracted by this procedure.

An experiment was conducted to determine if coumarin in filler will migrate into CA filters. The filter was removed from a L.S. Filter cigarette and a new 20 mm Marlboro filter was attached. The L.S. Filter rod was chosen because of the high level of coumarin in the filler. The filter plugs were analyzed after 1, 2, and 3 weeks and levels of 6, 7, and 8 ug/plug respectively were found. These results indicate that coumarin will drift into the filter but does not account for the high levels found in L.S. Filters.

D. Recommended Procedure

The recommended procedure shown in the Appendix will be placed in the Analytical Services Division Methods Manual.

III. REFERENCES

1. Feigl, Fritz, Spot Tests in Organic Analysis, 464-466 (1960).
2. Guenther, Ernest, The Essential Oils, 2, 659-663 (1952).
3. Notebook 3137, pp 1-48.

TABLE I

<u>Philip Morris</u>	<u>Date of Manufacture (1965)</u>	<u>ug Coumarin in Filler Rod</u>
P.M. Commander	June	51
Parliament	June	46
Alpine	June	11
Marlboro	June	14
P.M. Filter	April	13
<u>Reynolds</u>		
Winston (80's)	March	not detected
Salem	April	not detected
Camels	June	not detected
<u>American</u>		
Pall Mall	April	46
Tareyton	April	55
L.S. Filter	June	182
<u>Liggett and Myers</u>		
L and M (80)	March	22
Lark	June	153
<u>P. Lorillard</u>		
Kent (80)	April	41
<u>Brown and Williamson</u>		
Viceroy (80's)	March	not detected
Kool Filter	June	28
Bel Air	June	27